IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No. 9200
Brian Wong et al.	Examiner: Joseph F. Murphy
Application No.: 10/039,761	Technology Center/Art Unit: 1646
Filed: October 19, 2001	DECLARATION UNDER 37 C.F.R. § 1.131 OF C. ALAN FU, HELENA MANCEBO, BRIAN WONG AND XIULAN ZHOU
For: MODULATORS OF LEUKOCYTE ACTIVATION, COMPOSITIONS AND METHODS OF USE	
Customer No.: 20350	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, C. Alan Fu, Helena Mancebo, Brian Wong, and Xiulan Zhou, were at the time of the invention employed by Rigel Pharmaceuticals, Inc., the assignee of the above-referenced patent application. We are the named and true inventors of the subject matter disclosed and claimed in the above-referenced patent application.

The present invention provides methods of screening for bioactive agents that modulate USP-25 (also referred to as SUP) activity by combining a USP-25 protein, a USP-25 target protein, and a candidate bioactive agent; and then determining the level of ubiquitin-conjugated or ubiquitin-like protein-conjugated target protein in the presence and absence of the candidate bioactive agent. The invention is based, at least in part, on the identification of USP-25 as a ubiquitin peptidase (also referred to as a ubiquitin-specific protease or a ubiquitin isopeptidase) that is widely expressed in human tissues.

We conceived of and reduced to practice the claimed invention in the United States prior to December 28, 2000. The attached Exhibit B provides evidence of the conception of the invention and its reduction to practice. Exhibit B, with dates redacted therefrom, includes an invention disclosure prepared prior to December 28, 2000, which describes experimental work done prior to December 28, 2000. This work was done by us or under our supervision.

EXHIBIT A

Exhibit B describes the experimental work of Example 1-4 and Figures 5-7 and 9 bottom of the above-referenced patent application. The experiment described by Example 1, Figure 2 of Exhibit B, shows the sequence of USP-25 and identifies ubiquitin protease and hydrolase domains. The experiment also identifies conserved residues, *i.e.*, cysteine and histidine residues, of USP-25 that we believed were important for its function as a ubiquitin peptidase. Figure 4 of Exhibit B (containing the same data as Example 3 and Figure 6 of the present application) shows that mutation of the conserved cysteine residue of USP-25 affects the activity of the protein *in vivo*, demonstrating that ubiquitination activity is indeed important for the biological function of USP-25. Figure 5 of Exhibit B (containing the same data as Example 4 of the present application) shows that USP-25 and Syk associate *in vivo*. Figure 6 of Exhibit B (containing the same data as Figure 7 of the present application) provides a model for the interaction of USP-25 and Syk in B cells. In addition, Figure 3 of Exhibit B (containing the same data as Example 2 and Figure 9 bottom of the present invention) demonstrate that USP-25 is expressed in many human tissues in addition to B cells, and thus is likely active in ubiquitination of many non-B cell specific proteins. The description and Figures of Exhibit B demonstrate that we identified USP-25 as a ubiquitin peptidase, and moreover, provide teachings for those of skill on how to assay activity of USP-25 and thus, to identify modulators of USP-25 activity as is claimed. Therefore, the experiments described and presented in Exhibit B demonstrate conception and reduction to practice of the claimed methods.

In view of the foregoing, we respectfully submit that the evidence provided in Exhibit B unequivocally establishes that the claimed invention was conceived of and reduced to practice prior to December 28, 2000.

We further declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

The Declarants have nothing further to say.

Dated: 12/8/04

C. Alan Fu

Dated: _____

Helena Mancebo

Dated: _____

Brian Wong

Dated: _____

Xiulan Zhou

Attachments
BLK:blk
60341815 v1

We further declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

The Declarants have nothing further to say.

Dated:_____

C. Alan Fu

Dated: 12-10-2004

Helena Mancebo

Dated:_____

Brian Wong

Dated:_____

Xiulan Zhou

Attachments
BLK:blk
60341815 v1

We further declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

The Declarants have nothing further to say.

Dated:_____

C. Alan Fu

Dated:_____

Helena Mancebo

Dated: 12/13/04

Brian Wong

Dated: 12/1/04

Xiulan Zhou

Attachments
BLK:blk
60341815 v1

#102

INVENTION DISCLOSURE FORM

CONFIDENTIAL

Title of Invention:

I. Proposed Contributions:

SUP (Syk interacting ubiquitin protease) (B cell related)

II. Invention Statement (attach additional pages with exhibits as needed):

SUP is a novel protein that was identified as a Syk interacting molecule in the yeast two-hybrid system (Figure1). Full-length sequence of SUP was obtained by RT-PCR and 5′ race studies. Bioinformatic analysis revealed that its sequence shared significant homology with ubiquitin proteases or isopeptidases that removed ubiquitin from their substrates. SUP has a cystein and two histidine residues that are conserved within the catalytic domains of the ubiquitin protease family (Figure 2). We isolated two isoforms of SUP from the library, possibly derived from alternative splicing. Northern blot analysis showed that SUP was ubiquitously expressed in all tissues tested, including spleen and thymus (Figure 3).

We generated catalytically dead mutant from of SUP (mt SUP) by mutating the catalytic cystein residue into serine (Figure 2). In a transient transfection assay in B cells, mt SUP inhibited anti-IgM induced NFAT-luciferase activity, acting as a dominant negative mutant (Figure 4). This result strongly suggests that SUP is a positive regulator of the BCR-mediated signaling pathway. In addition, Syk and SUP are indeed associated with each other when co-overexpressed in Jurkat cells (Figure 5). Collectively, SUP could function in the BCR signaling pathway by removing ubiquitin from Syk and therefore stabilizing Syk, which is a key B cell regulator in the antigen receptor signaling pathway (Figure 6).

Our findings strongly suggest that SUP could be used as a cellular target for developing specific pharmacological agents that inhibit B cell functions. Any small molecule or peptide that inhibits the function of SUP could function as a B cell immunosuppressant and be used for therapeutic treatment of immunological related diseases, such as autoimmune diseases and transplantation rejections.

III. Priority Data:

A. Earliest Date and Location of Record of Invention:

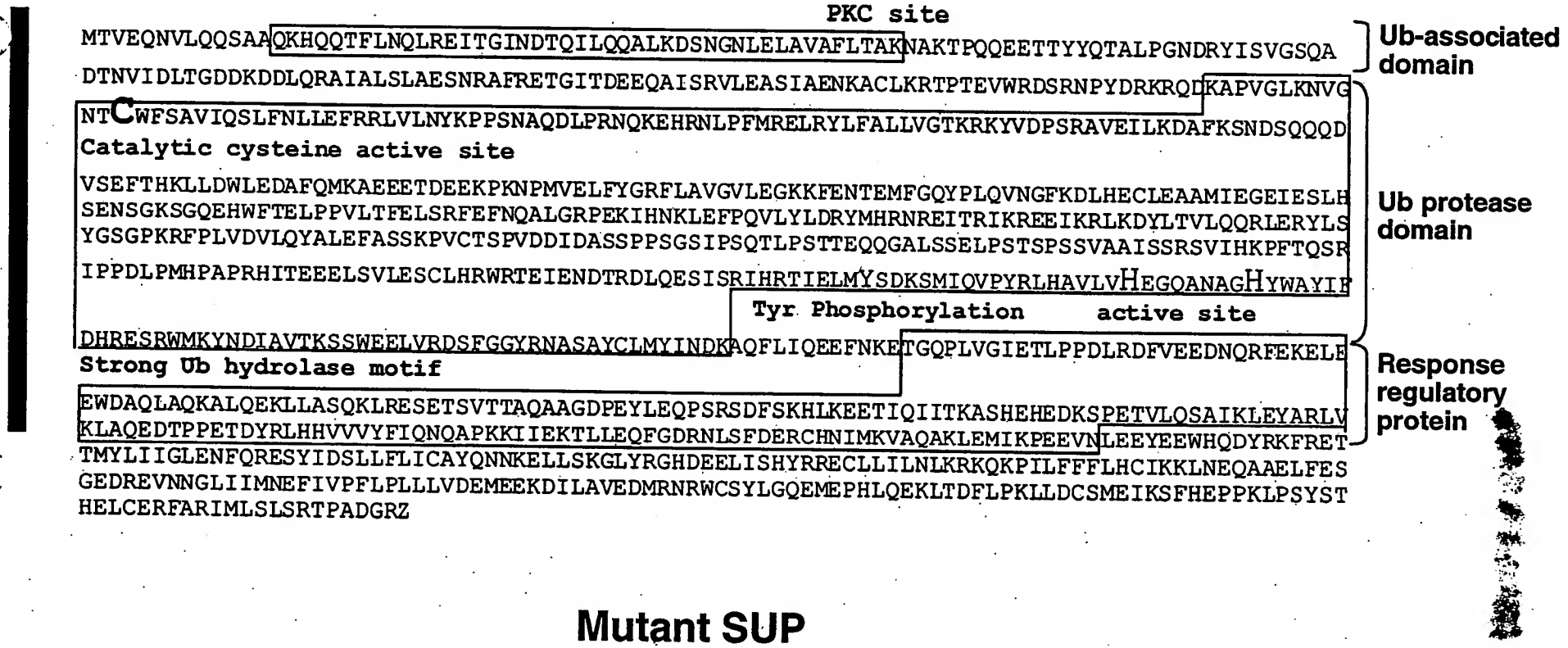
The first functional data showing that SUP played regulatory role in the BCR signaling pathway was recorded on page 163 in Rigel note book #188 (Xiulan Zhou), on [redacted]

Inventor Initials _____ Date _____

EXHIBIT B

Figure 2. Sequence of SUP

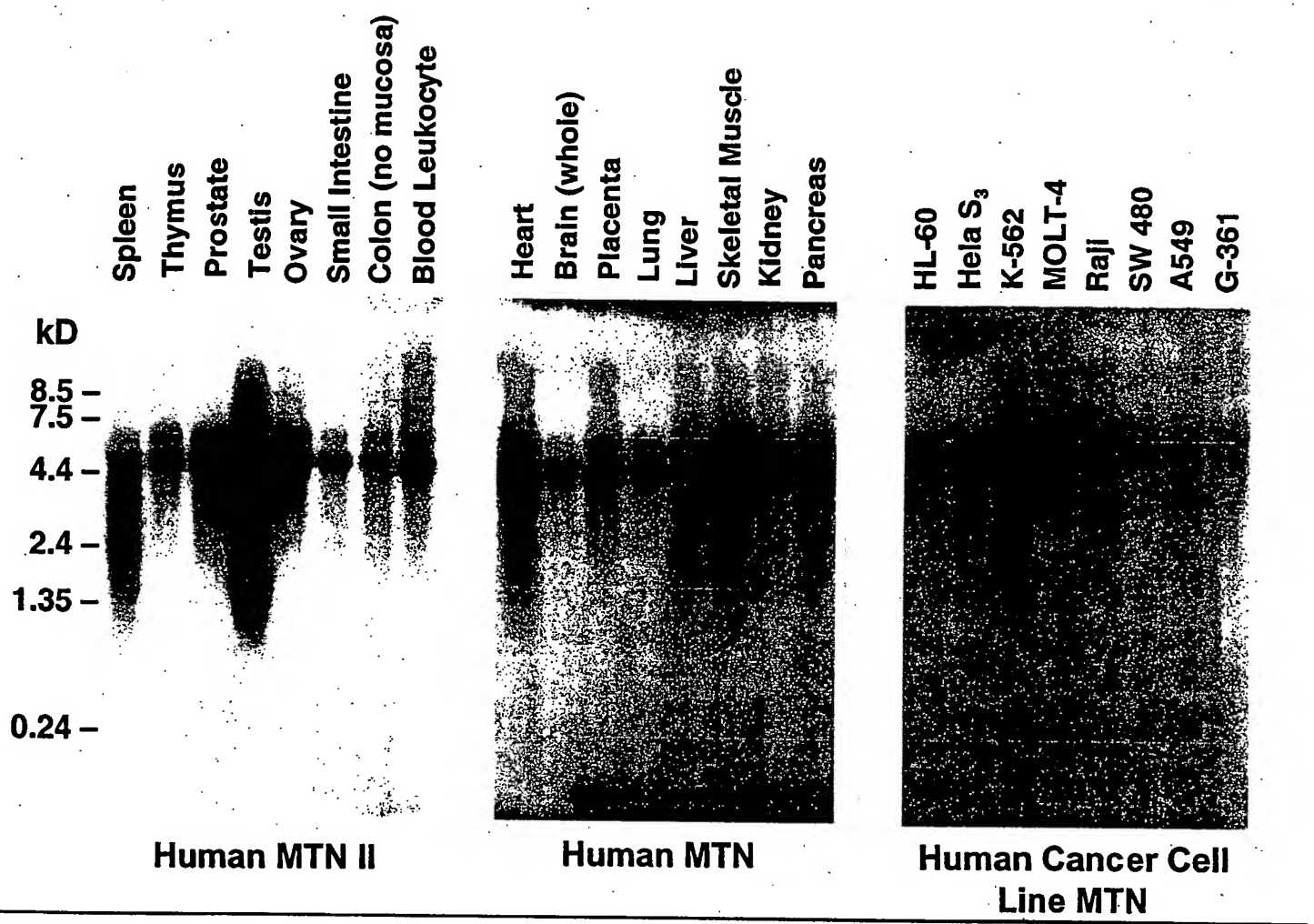
Wild type SUP



Mutant SUP

Cys → Ser

Figure 3. Northern Blot Analysis of SUP



- SUP is ubiquitously expressed.
- The low level of SUP expression in spleen may render it more sensitive to inhibitory pharmacological agents.

Figure 4. mtSUP Suppresses α-IgM Induced NFAT-Luciferase Activity as a Dominant-negative Mutant

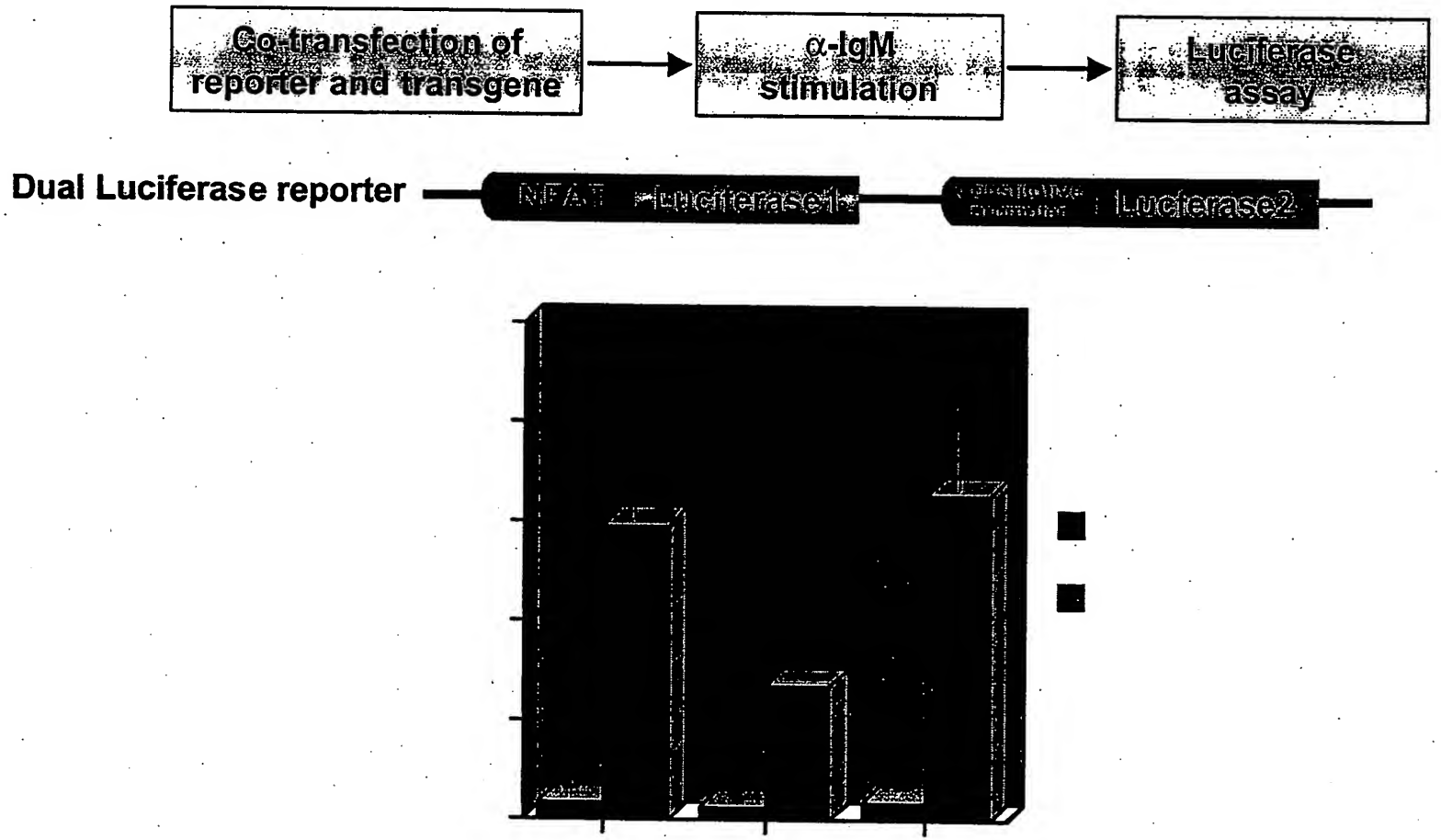
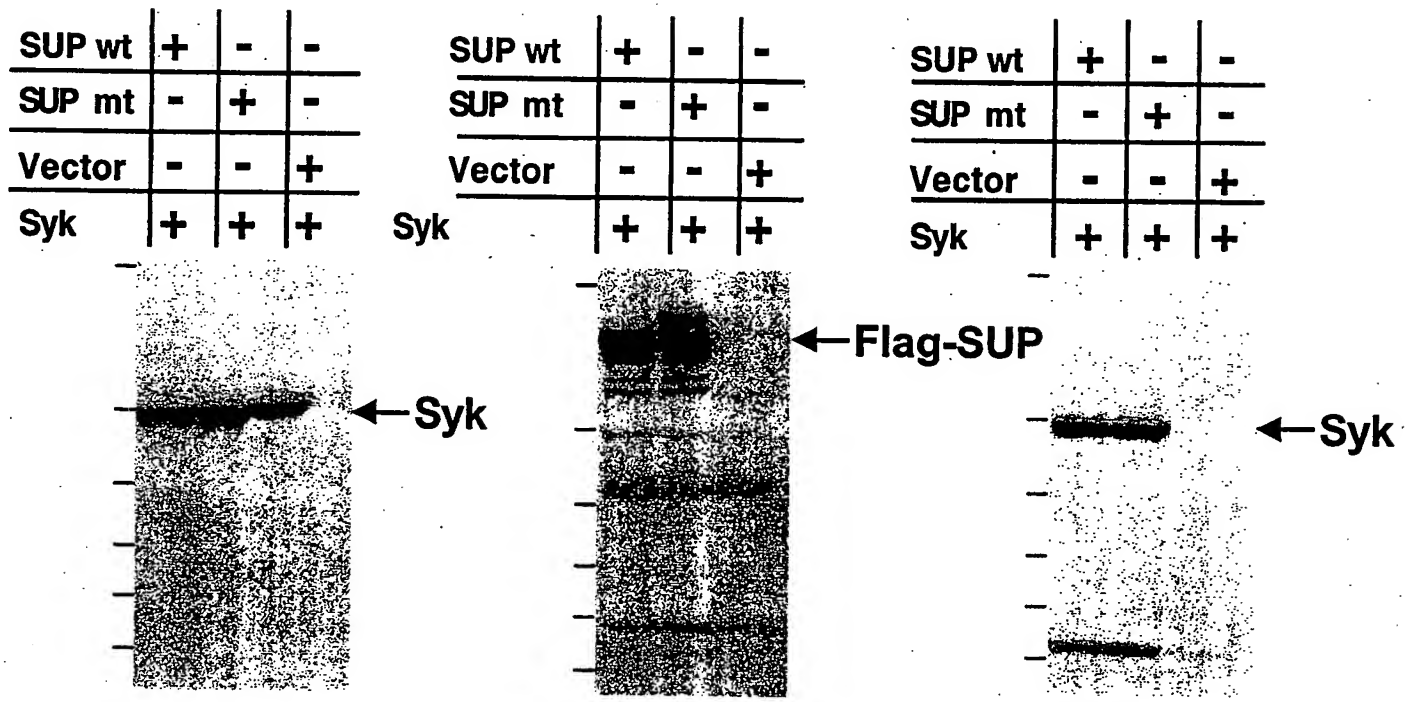


Figure 5. Association of SUP and Syk when Over-expressed in Jurkat Cells



Flag-tagged wild-type and catalytically-inactive mutant SUP both interact with Syk in transiently transfected Jurkat-LgT cells

Figure 6. Model: SUP Regulates BCR Signaling by Stabilizing Syk